

Fig. 1A

Fig. 1C

Fig. 1E

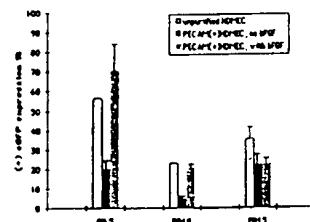
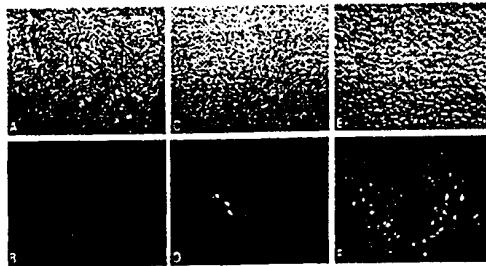


Fig. 1B

Fig. 1D

Fig. 1F

Fig. 2



Fig. 3A

Fig. 3B

Fig. 4A

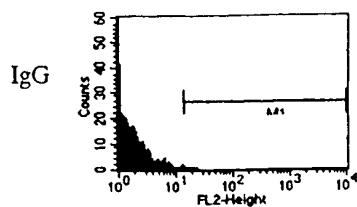


Fig. 4B

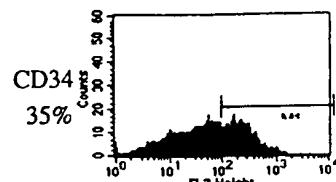
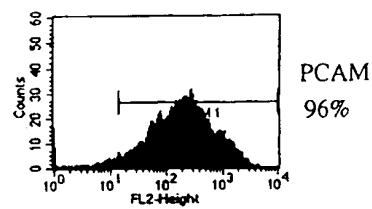


Fig. 4C

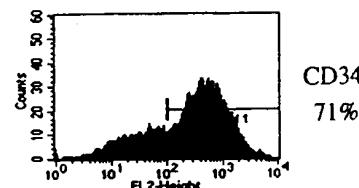


Fig. 4D

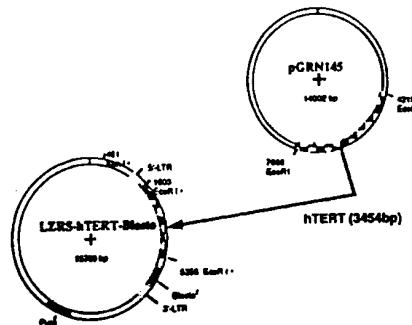


Fig. 5

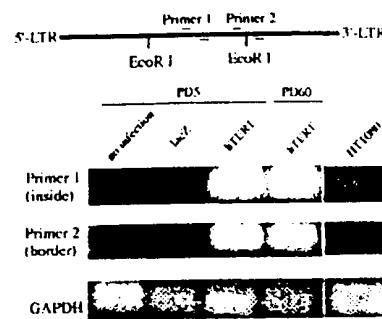


Fig. 6

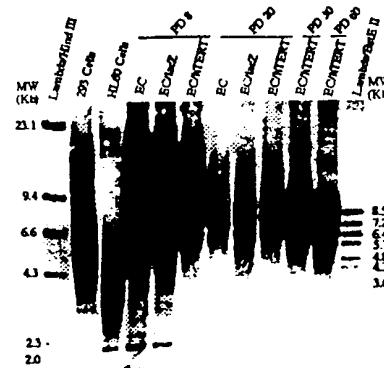


Fig. 7

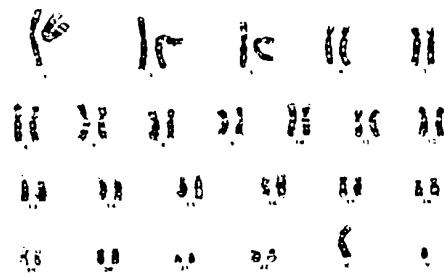


Fig. 8

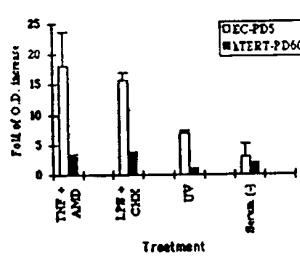


Fig. 9A

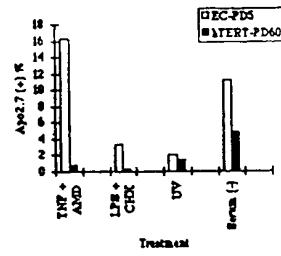


Fig. 9B

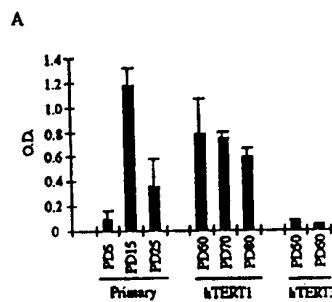


Fig. 10A

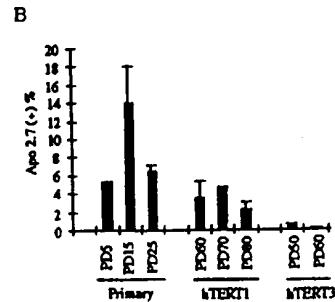
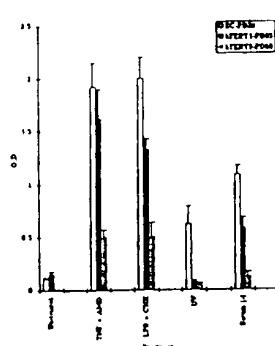
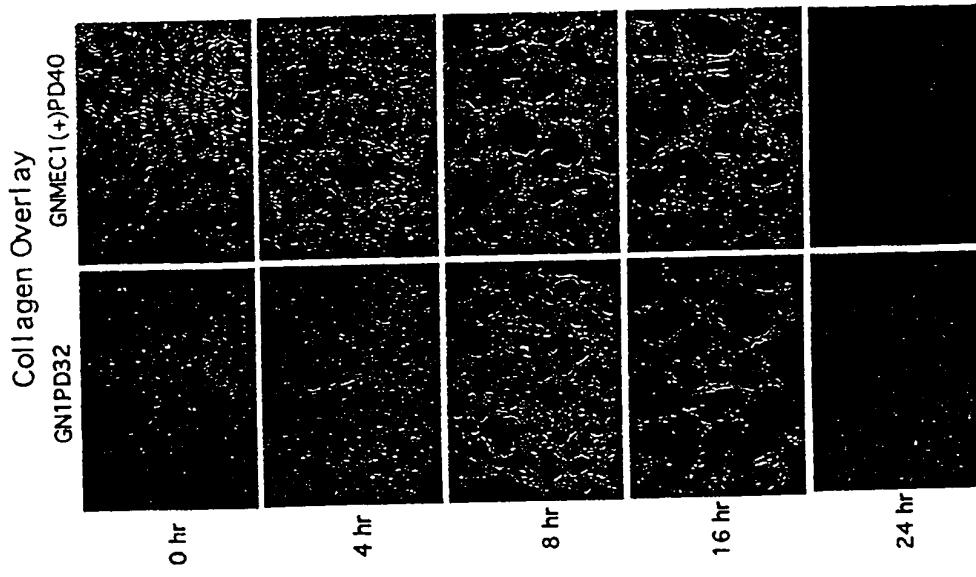


Fig. 10B





**Figure 14**

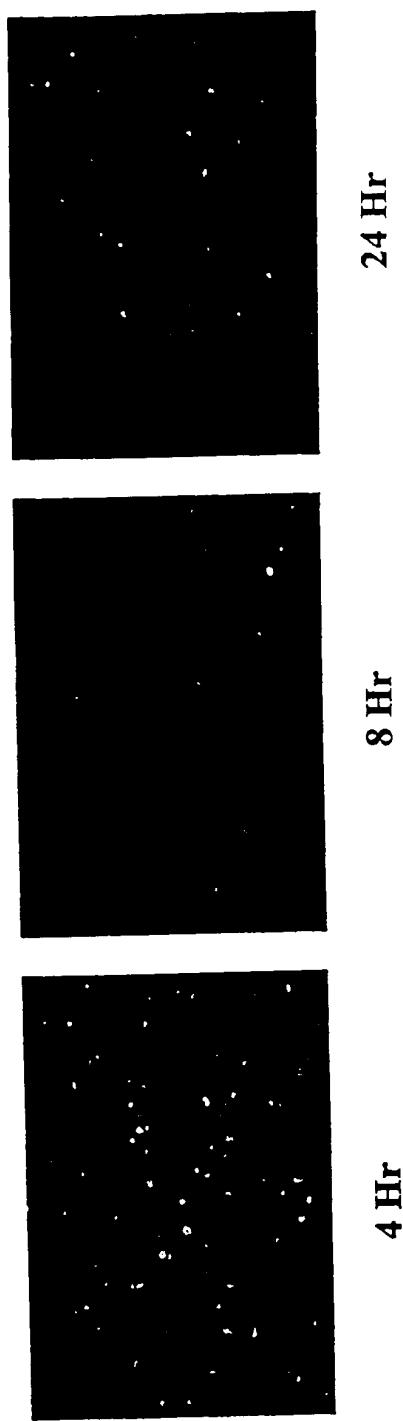
Time course of apoptotic induction by 3D collagen overlay *in vitro* and superior survival of Tert-EC versus primary parental EC. By 16hr after collagen overlay, parental cultures are undergoing dissolution and apoptosis whereas, Tert-EC are still forming tubule structures. At 24hr, all parental cells are dead and Tert-EC are stable.

GN1PD32: eGFP-labeled primary HDMEC at PD32 (mid-late passage)

GNMEC1(+)PD40: hTERT(+) GNMEC-1 at PD40

**Figure 15**

**Time Course of High Density Matrigel Tubule Formation**



Demonstration of the utility of using eGFP-labeled Tert-EC for tracking morphogenetic patterns of cells forming microvascular structures *in vitro*

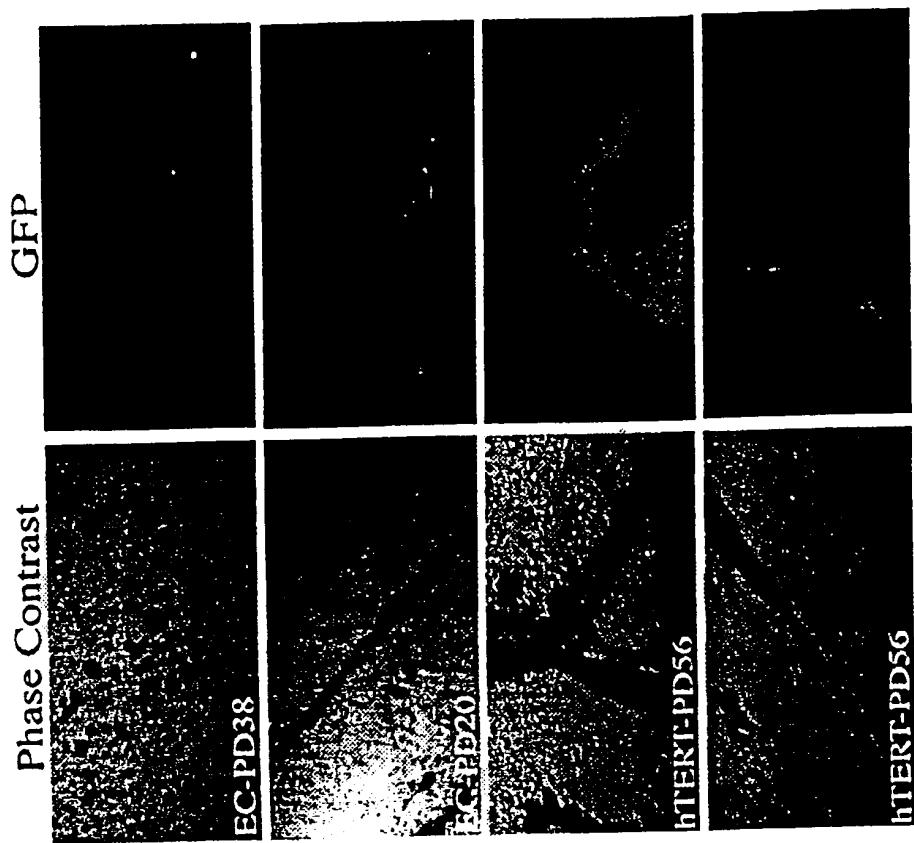


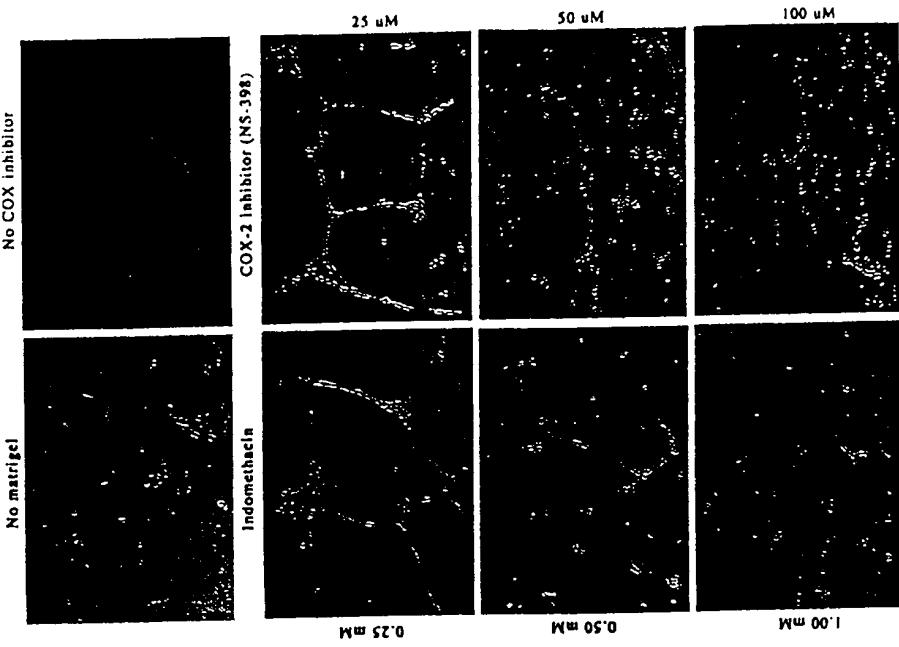
Figure 16

Superiority and utility of eGFP-labeled Tert-EC in formation of vascular structures in 3D Matrigel *in vitro*.

Senescent parental EC (EC-PD38) show no vessels in 3D Matrigel compared to younger cells (EC-PD20). Vessels were numerous and prominently labeled in all Tert-EC cultures (hTERT1-PD56).

Figure 17

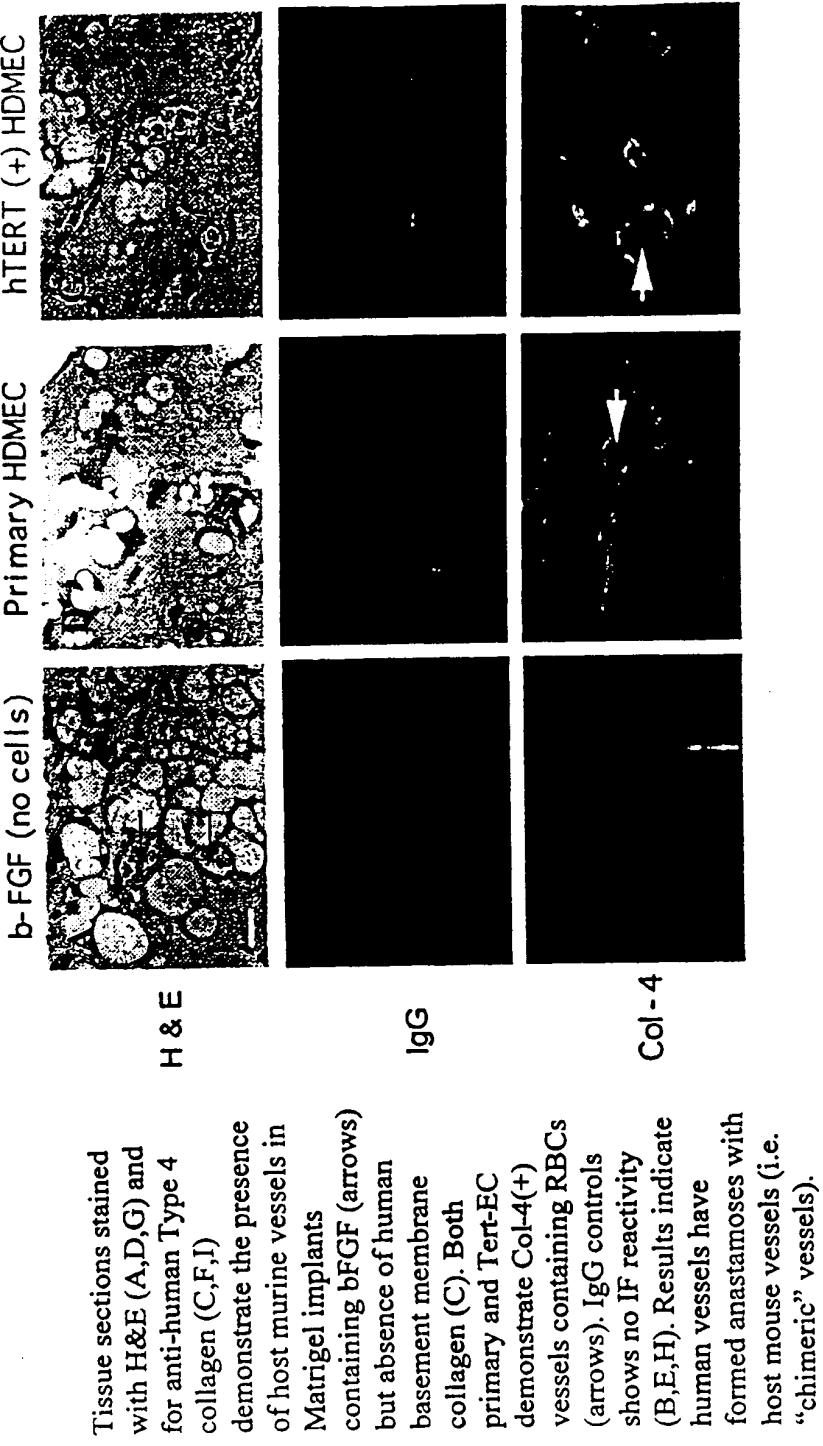
Inhibition of *in vitro* angiogenic web formation by cyclo-oxygenase (COX) antagonists



Tert-EC were incubated in the presence of two different COX inhibitors (indomethacin and NS-398) at different concentrations and examined 12hr after plating on Matrigel. Controls show no vessels in absence of Matrigel and numerous vessels in absence of or at low dose COX blockers. Graded inhibition of vessel formation is observed with increasing doses of COX blockers, with the COX-2 specific inhibitor (NS-398) 10-fold more potent than the general inhibitor, indomethacin.

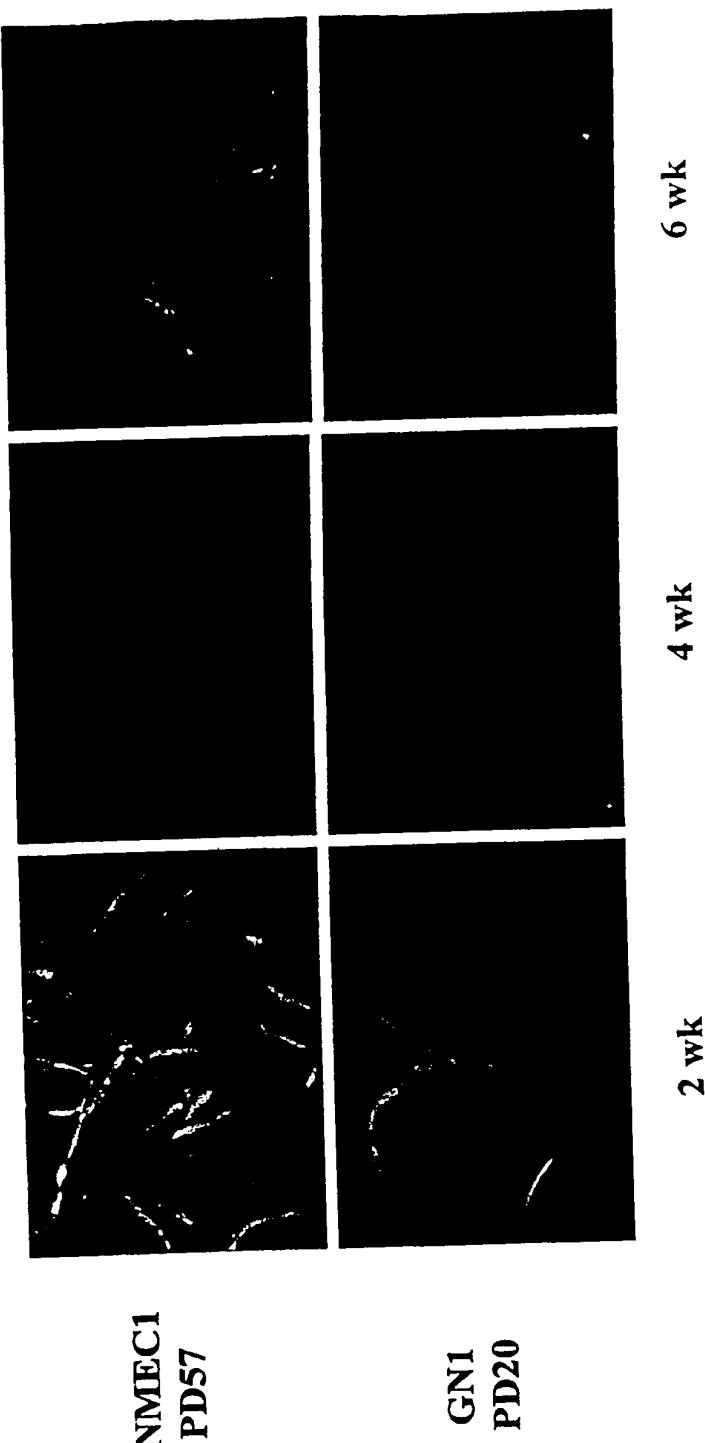
**Figure 18**

**SCID-Mouse Matrigel Implantation of Human HDMEC**



## Figure 19 Superiority of Tert-EC at In Vivo Microvessel Formation

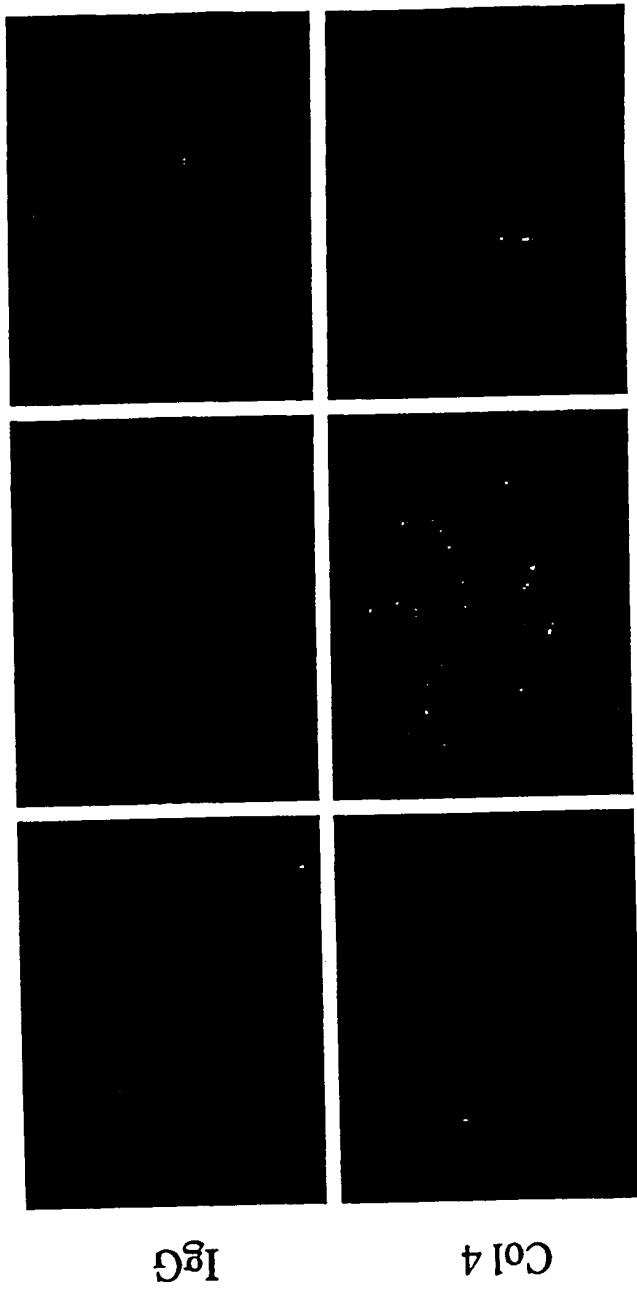
Early-mid passage parental eGFP-labeled HDMEC (GN1-PD20) were directly compared to Tert-EC line (GNMEC-1) for their ability to form fluorescent microvessels *in vivo* using the SCID mouse Matrigel implantation assay system. Tert-EC demonstrated more fluorescent vascular structures at all time points after implantation versus parental line.



**Figure 20** Superiority of Tert-EC at In Vivo Microvessel Formation

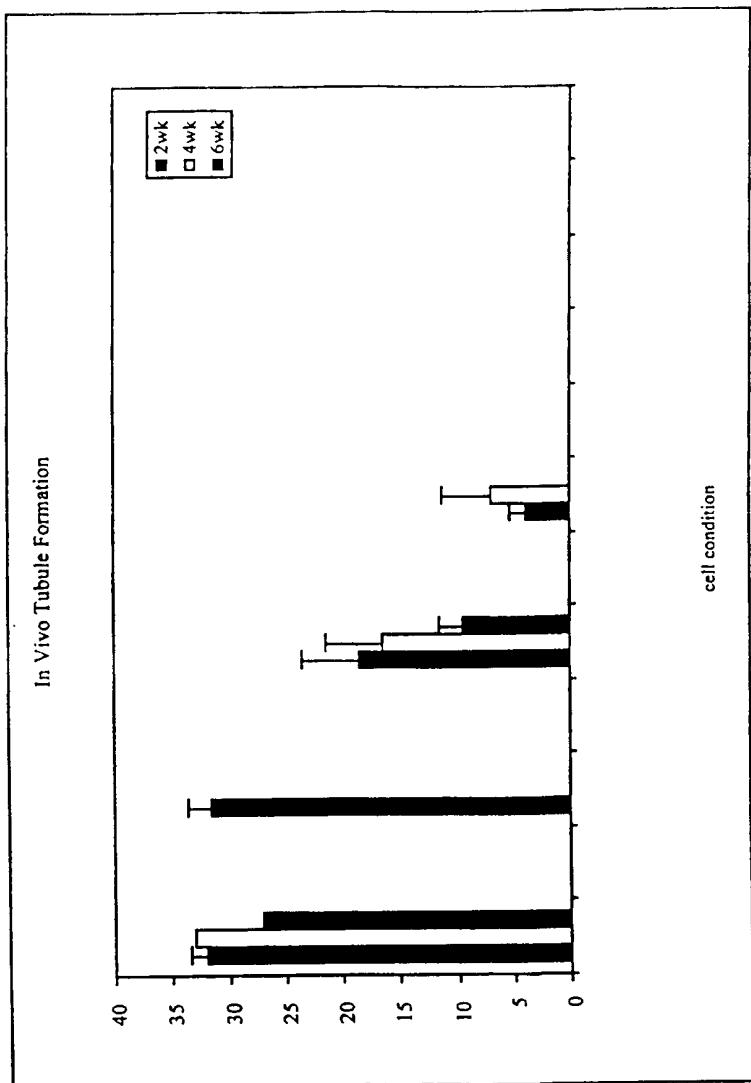
Use of Type 4 collagen IF reactivity to quantify human microvessels *in vivo* demonstrates superior durability of Tert-EC (GNMEC1) versus late passage parental cells (GN1PD34). Younger parental cells (PD20) show many small diameter vessels.

GN1PD34  
GN1PD20  
GNMEC1PD57



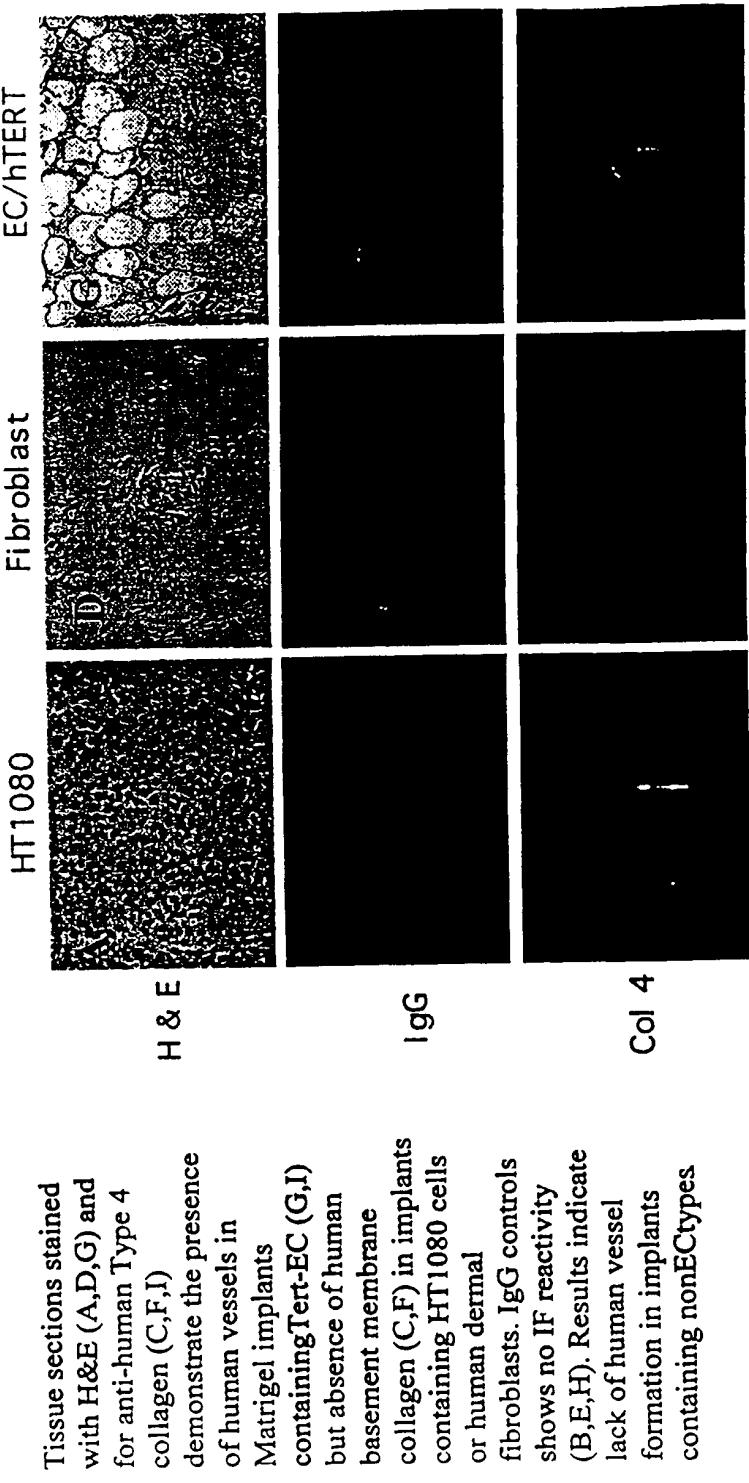
## Figure 21 Superiority of Tert-EC at In Vivo Microvessel Formation

Quantitative comparison of microvessel density by IF-micromorphometry using anti-human Type 4 collagen demonstrates equal number of vessels in young primary (GN1PD12) vs Tert-EC (GNM/EC1) and maintenance of vessels at 2, 4 and 6 weeks after implantation in Tert-EC ( $>25$ ); whereas, mid and late passage parental EC show both decreased numbers and loss of vessels with time *in vivo*. Dermal fibroblasts (Fb) and human fibrosarcoma cells (HT-1080) show no vessel formation (see Figures 22, 23).



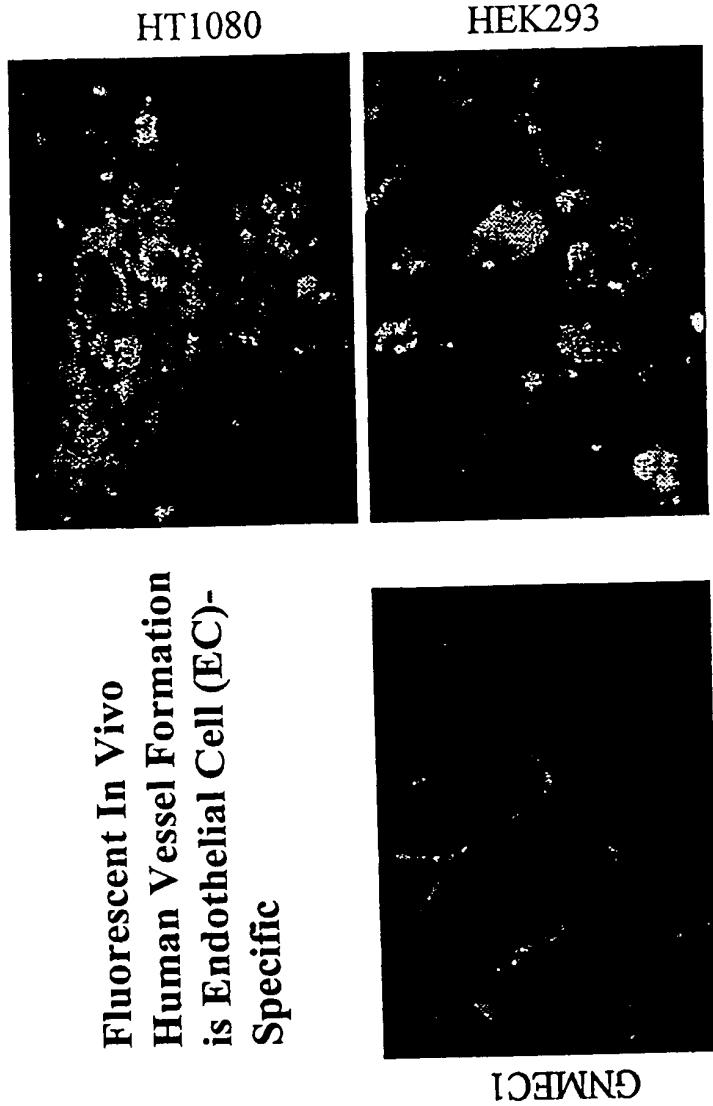
## Figure 22

### Specificity of Tert-EC at In Vivo Microvessel Formation



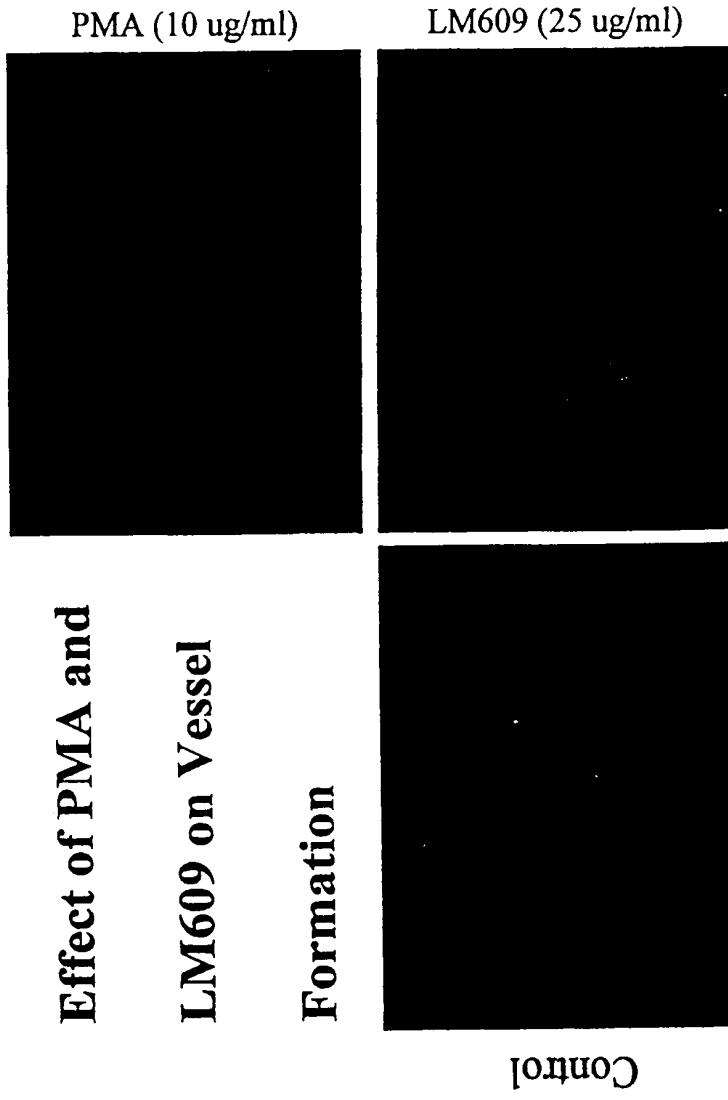
**Figure 23**

**Fluorescent In Vivo  
Human Vessel Formation  
is Endothelial Cell (EC)-  
Specific**



Human fibrosarcoma cells (HT1080) and human embryonic kidney tumor cells (HEK293) expressing eGFP show fluorescent tumor masses but no microvessels, whereas, Tert-EC (GNMEC1) form obvious fluorescent vessels.

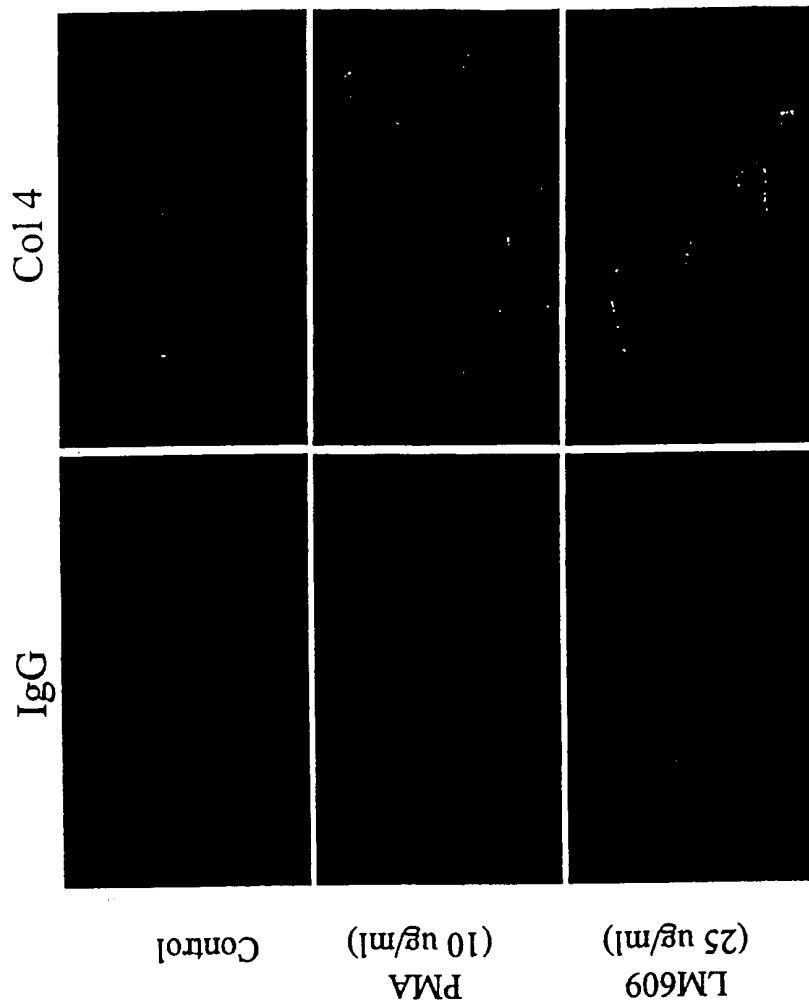
**Figure 24**



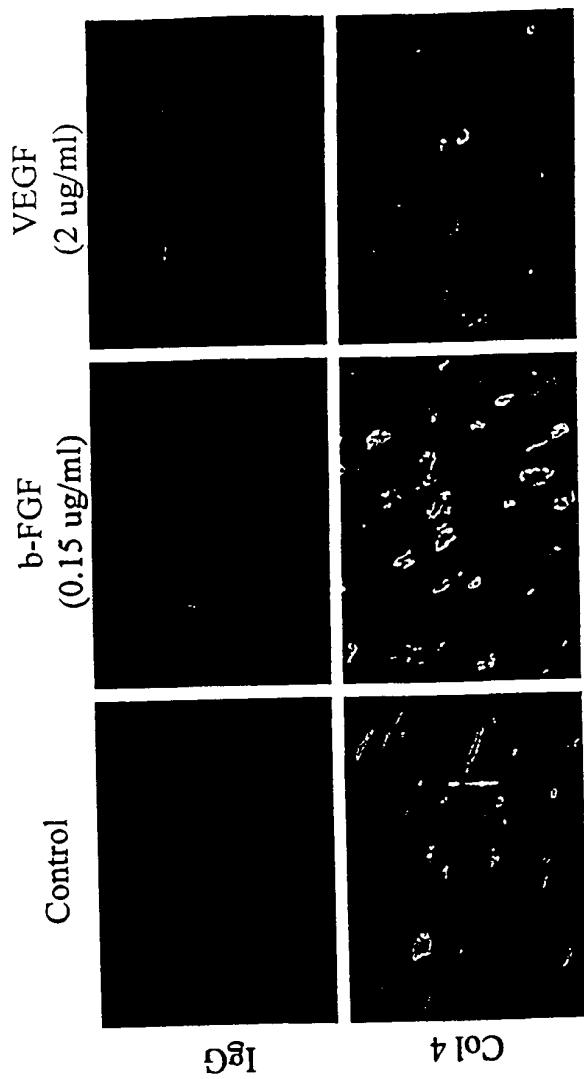
Treatment of Tert-EC with phorbol ester (PMA) or vitronectin receptor ( $\alpha_v\beta_3$ )-antagonist (LM609) for 2 hrs prior to implantation *in vivo*. Results show PMA has a slight negative effect on vessel density, whereas, LM609 appears to increase vessels relative to control, untreated cells.

## Figure 25

Effect of PMA and LM609 on *In Vivo* Vessel Formation

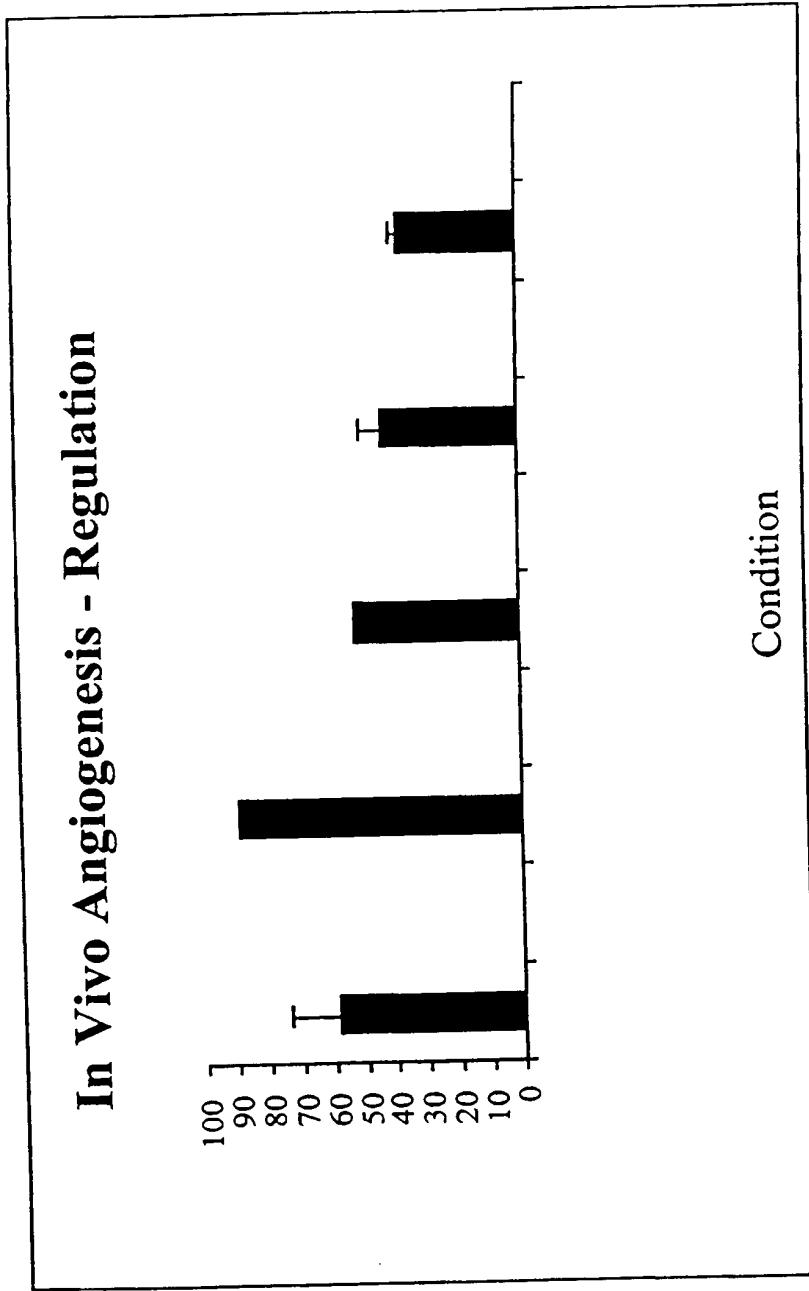


Quantification of vessel density by IF staining of human Type 4 collagen shows effects of PMA and LM609, as described in Figure 24.

**Figure 26****Effect of Growth Factors on Vessel Formation**

Quantification of vessel density by IF staining of human Type 4 collagen shows effects of b-FGF and VEGF. Results indicate FGF increases vessel density, whereas, VEGF has little effect.

**Figure 27**



Effect of different angiogenic regulators on GNMEC1 *in vivo* microvessel formation. Results show significant changes in vessel density only by b-FGF.



FIGURE 29

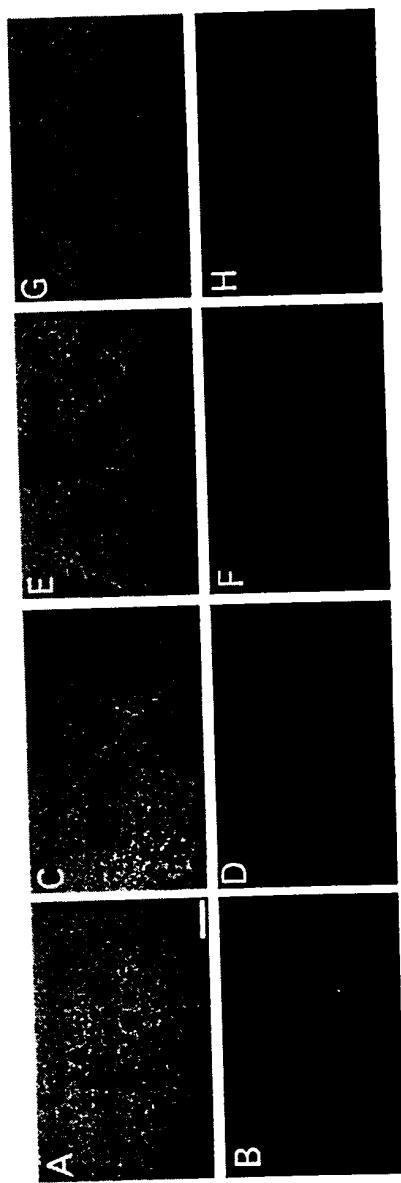
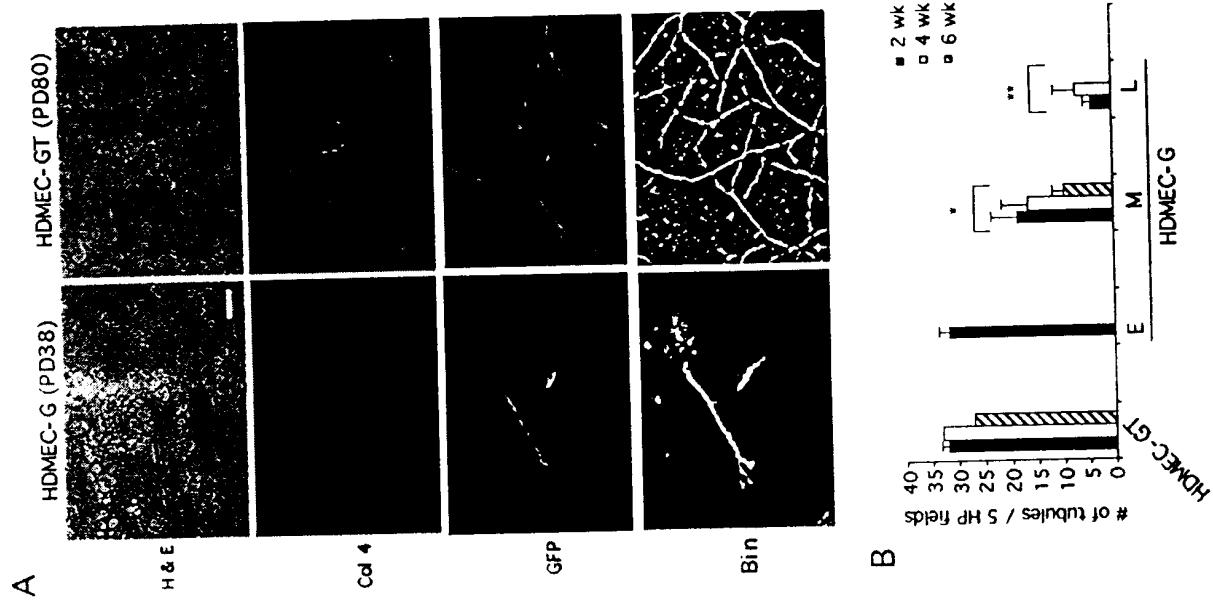


FIGURE 30



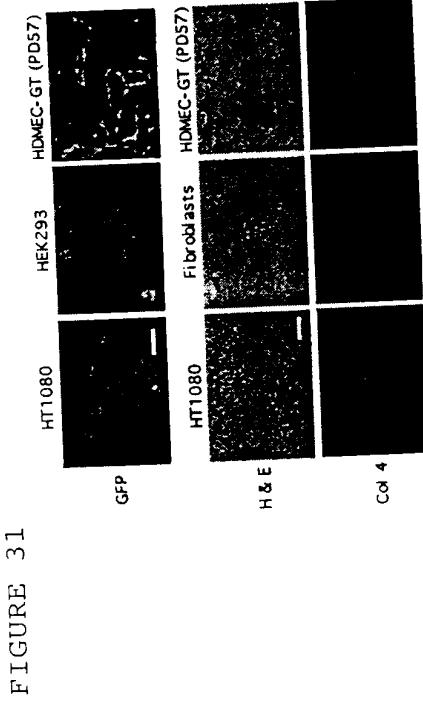
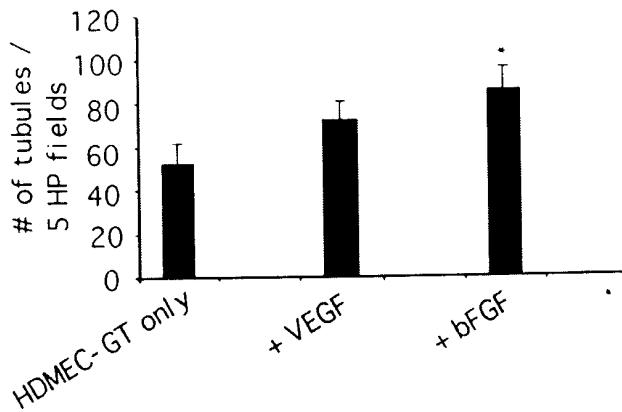
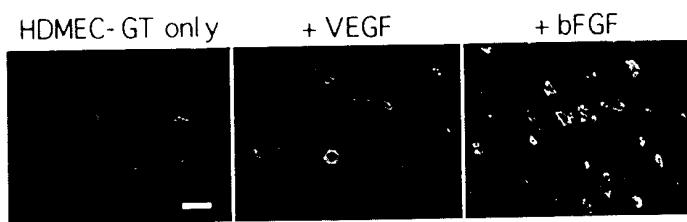


FIGURE 33

A



B

